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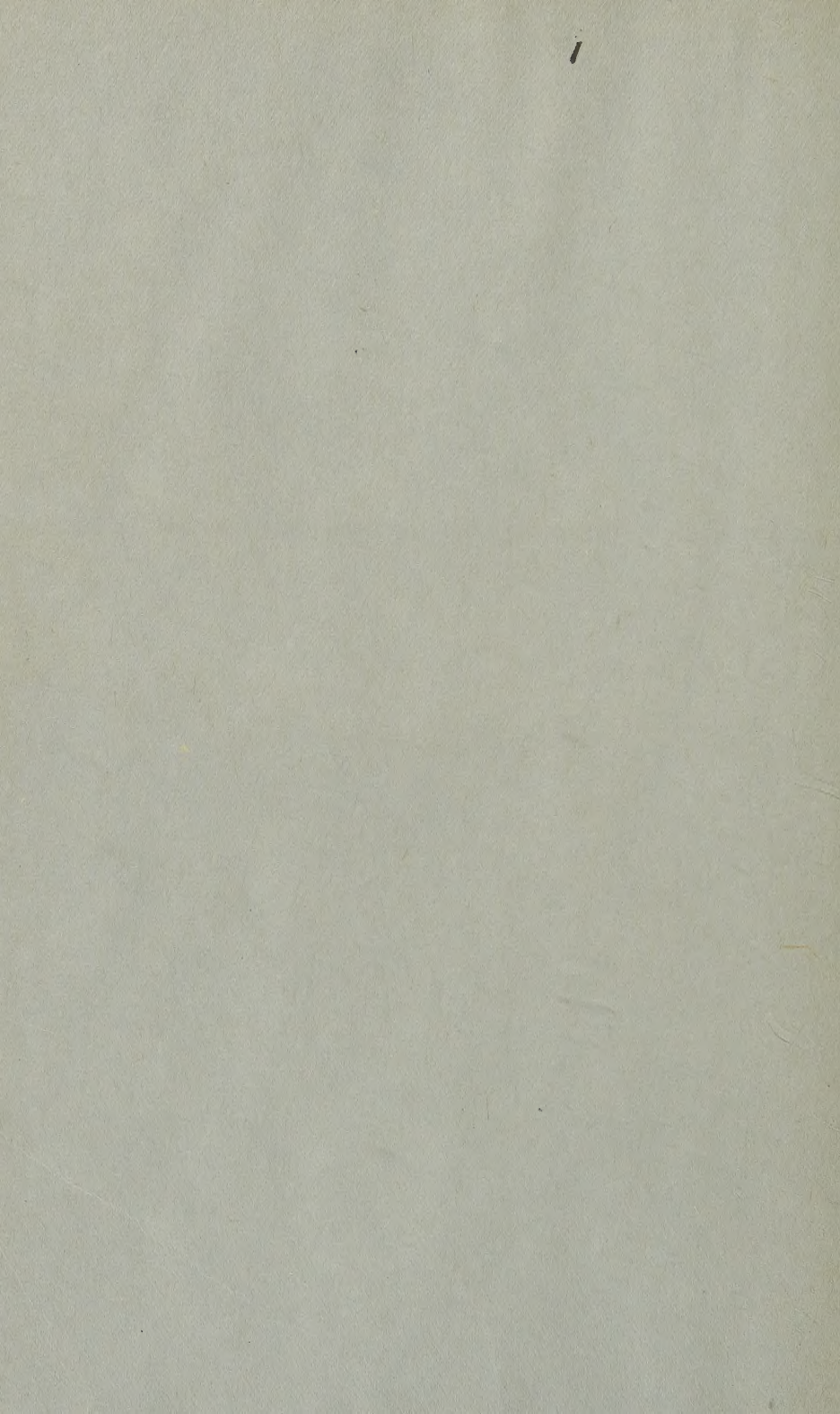
A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
THE UNIVERSITY OF MICHIGAN.

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THE METABOLISM OF SULFUR.

XVII. THE RATE OF OXIDATION OF INGESTED CYSTINE IN THE ORGANISM OF THE RABBIT.

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(Received for publication, December 16, 1929.)

After oral administration, cystine is rapidly oxidized and its sulfur excreted by the kidneys, for the most part as inorganic sulfates. With the usual amounts fed, from 75 to 90 per cent of the sulfur present in the cystine molecule is excreted in oxidized form by the kidneys within the 24 hour period immediately following its ingestion. Oxidation of the sulfur after parenteral administration of cystine, particularly after its intravenous injection, does not appear to occur as readily as after feeding.

The experiments of Blum (1), in which cystine was administered orally and intravenously to dogs, are usually cited in this connection. Total sulfur and "labile" sulfur were determined in the urine and the excretion of cystine was considered as having been demonstrated if the sulfur split off as sulfide by boiling with alkali ("labile" sulfur) (2) showed an increase either in absolute amount or in relation to the amount of total sulfur. Large amounts of cystine (4.5 to 9.6 gm.) when fed were almost completely utilized or at least no significant increase in the "labile" sulfur of the urine was observed. Similar results were obtained with dogs poisoned with phosphorus. If however the cystine were introduced directly into the peripheral circulation (jugular or crural vein), cystine was excreted in the urine in such quantities that separation as the characteristic hexagonal plates occurred. When cystine was injected slowly into the mesenteric vein, it could not be identified in the urine, nor was the "labile" sulfur increased significantly. Blum suggested that these differences in the utilization of cystine after introduction into the mesenteric and peripheral circulations

might have been due to a specific influence of the liver on cystine metabolism, possibly to the retention of cystine in the liver for synthesis of taurine. It should be pointed out however that the two series of experiments are not comparable, since in the peripheral intravenous injections, 1.4 and 1.5 gm. of cystine were injected into dogs of 9 and 13 kilos body weight, while in the other series, 1.4 gm. of cystine were introduced into the mesenteric veins of dogs of 30 and 31 kilos body weight. Thus the dose per kilo of body weight in the first series was more than double that of the second series. Moreover in none of the injection experiments was the total sulfur of the urine increased as compared with the normal days. Hence little evidence of the actual utilization or oxidation of the cystine is presented. This failure to observe any increase in the sulfur content of the urine after the injection of cystine is particularly significant in view of the more recent demonstration of the nephrotoxic action of cystine when administered in large amounts (3-7).

The present study was undertaken in an attempt to learn more about the mechanism of the oxidation of ingested cystine, the rate at which oxidation of the sulfur takes place, and the organs concerned in its metabolism. Cystine was given by mouth or subcutaneously to normal rabbits and the urine collected at 3 to 4 hour intervals and analyzed in order to determine the rate as well as the extent of oxidation, and the excretion of unchanged cystine. Similar studies carried out on rabbits whose livers had been injured by the injection of hydrazine will be reported in another paper.

EXPERIMENTAL.

Rabbits, maintained upon a stock diet of 50 gm. of oats and 75 gm. of cabbage daily with water *ad libitum*, received cystine or an ester of cystine either by mouth or subcutaneously. The cystine was usually given at 8 a.m. and the day's food supply was placed in the cage at 5 p.m. This procedure gave ample time for the absorption of cystine from the alimentary tract. The urine was removed by gentle pressure on the abdominal wall. The urine was collected in varying periods as indicated in Tables I to III.

Sulfur partitions were made according to the benzidine volumetric method of Fiske (8), with the use of Jena glass filter

crucibles (No. 10G 3/<7) instead of the paper pulp filters recommended by Fiske. Nitrogen was estimated by the Gunning modification of the Kjeldahl method, creatinine by Folin's micro method, and cystine by the colorimetric method of Looney (9). Okuda (10), who has made a critical study of the Looney method for cystine as applied to urine, concluded that "sodium sulphite itself reacts with the uric acid reagent to give a faintly blue color, and some urinary constituents other than uric acid give a different intensity of color whether in the presence or absence of sodium sulphite. Consequently Looney's method gives higher results especially in urines poor in uric acid." Looney's method according to Okuda gave cystine values from 3 to 10 times as high as those obtained on the same urine samples by Okuda's micro iodine method, the differences not being constant in the various urine samples studied. The cystine values presented in our Tables I to III may, therefore, be higher than the true values, since rabbit urine does not contain any significant amounts of uric acid.

An examination of the data (Tables I to III) would indicate that three factors are significant in determining the rate of elimination of the products of cystine metabolism after its entrance into the system; *i.e.*, degree of absorption, rate of oxidation, and rate of excretion by the kidneys. The amount of absorption in a given period, particularly after subcutaneous injection of cystine, would appear to vary considerably. Although the solutions of cystine injected were always carefully neutralized, in some cases marked edema was produced at the site of injection within a few hours. For this reason oral administration gave most satisfactory results.

In the experiments with Rabbit 14 (Table I), cystine was first given by subcutaneous injection and was evidently very rapidly absorbed from the site of injection, so that the oxidative capacity was exceeded and unchanged cystine was eliminated by the kidneys. 3 hours after the injection, the urine obtained from the bladder contained a heavy precipitate of cystine crystals, and on analysis was shown to contain 137.8 mg. of cystine. An oliguria then developed despite the fact that water was given by stomach tube. As a result, there was not only a failure to excrete the products of oxidation of the ingested cystine, but the normal

TABLE I.
Partition of Sulfur in Urine after Cystine Administration.

Rabbit 14; weight 2.6 kilos.

Day of experiment.	Length of period.	Creatinine.	Total S.	Total SO ₄ -S.	Inorganic SO ₄ -S.	Ethereal SO ₄ -S.	Organic S.	Cystine.	Cystine S.
	hrs.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	3		16.8	12.5	11.2	1.3	4.3		
	3		10.6	7.9	7.9	0.0	2.7		
	18		93.0	78.6	71.0	7.6	14.4		
	24		120.4	99.0	90.1	8.9	21.4		
2	3		9.3	7.1	6.4	0.7	2.2		
	3*		76.8	18.7	18.6	0.1	58.1	137.8	36.8
	6		5.8	1.5			4.3	11.2	3.0
	12†		39.7	31.0	30.7	0.3	8.7	16.1	4.3
	24		131.6	58.3			73.3		
3‡	7		47.6	43.6	42.2	1.4	4.0	8.9	2.4
	17		203.0	181.7	175.7	6.0	21.3	24.6	6.6
	24		250.6	225.3	217.9	7.4	25.3	33.5	9.0
4	6	28.6	13.1	9.7	5.5	4.2	3.4	8.6	2.3
	18	82.4	113.2	102.2	89.9	12.3	11.0	18.0	4.8
	24	111.0	126.3	111.9	95.4	16.5	14.4	26.6	7.1
5	6	27.8	21.4	16.0	14.8	1.2	5.4	7.7	2.0
	18	62.5	107.7	93.6	86.3	7.3	14.1	15.7	4.2
	24	90.3	129.1	109.6	101.1	8.5	19.5	23.4	6.2
6	6	27.8	21.4	16.4	14.8	1.2	5.4	7.7	2.0
	18	62.5	107.7	93.6	86.3	7.3	14.1	15.7	4.2
	24	90.3	129.1	109.6	101.1	8.5	19.5	23.4	6.2
7	6§	40.4	123.4	114.6	104.4	10.2	8.8	12.9	3.4
	6	22.5	156.4	141.4	137.4	4.0	15.0	14.3	3.8
	12	53.0	261.4	242.1	233.8	8.3	19.3	12.4	3.3
	24	115.9	541.2	498.1	475.6	22.5	43.1	39.6	10.5
8	6	19.1	40.0	33.6	32.2	1.4	6.4	5.7	1.5
	18	93.0	135.5	112.1	104.6	7.5	23.4	24.8	6.1
	24	112.1	175.5	145.7	136.8	8.9	29.8	30.5	7.6
9	24		166.4	119.5	112.5	7.0	46.9	25.0	6.7

* Cystine 1.94 gm. (sulfur = 0.517 gm.) given subcutaneously as the sodium salt; 50 cc. of water by mouth.

† Casts in urine. Urine volume very scanty (22 cc.). Kidney injury?

‡ The experiments were performed on consecutive days except that an interval of 50 days elapsed between the 3rd and 4th days designated.

§ Cystine 2.0 gm. (sulfur = 0.534 gm.) given by mouth as the sodium salt.

TABLE II.

Partition of Sulfur in Urine after Cystine Administration.

Rabbit 16; weight 2.75 kilos.

Day of experiment.	Length of period.	Total S.	Total SO ₄ -S.	Inorganic SO ₄ -S.	Ethereal SO ₄ -S.	Organic S.	Cystine.	Cystine S.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
1	3	10.7	9.5	7.1	2.4	1.2	6.9	1.9
	3	3.8		2.9			6.1	1.6
	18	123.6	111.1	100.9	10.2	12.5	12.5	3.3
	24	138.1		110.9			25.5	6.8
2	3	15.7	13.1	7.2	5.9	2.6	5.9	1.6
	3	9.1	8.3	6.1	2.2	0.8	4.1	1.1
	18	111.0	100.4	79.6	20.8	10.6	7.5	2.1
	24	135.8	121.8	92.9	28.9	14.0	17.5	4.8
3	3*	31.5	17.0	15.6	1.4	14.5	38.4	10.4
	3	71.2	48.9	41.5	7.4	22.3	48.3	12.9
	18	261.0	220.3	201.2	19.1	40.7	20.3	5.4
	24	363.7	286.2	258.3	27.9	77.5	107.4	38.7
4	3	21.7	17.4	15.9	1.5	4.3	6.8	1.8
	3	14.4	11.1	10.0	1.1	3.3	5.7	1.5
	18	108.2	94.4	87.8	6.6	13.8	19.5	5.2
	24	144.3	122.9	113.7	9.2	21.4	32.0	8.5
5	6	16.5	15.5	14.3	1.2	1.0	6.7	1.8
	18	130.4	113.4	107.3	6.1	17.0	23.1	6.2
	24	146.9	128.9	121.6	7.3	18.0	29.8	8.0
6	9	23.4	19.8	18.4	1.4	3.6	10.9	2.9
	15	113.1	99.6	96.0	3.6	13.5	15.5	4.1
	24	136.5	119.4	114.4	5.0	17.1	26.4	7.0
7	3	5.9		3.7			4.8	1.3
	6	18.5	14.2	12.6	1.6	4.3	7.9	2.1
	15	116.9	101.6	89.3	12.3	15.3	13.5	3.6
	24	141.3		105.6			26.2	7.0
8	3†	9.9	7.4	7.2	0.2	2.5	9.0	2.4
	3	26.3	22.8	20.9	1.9	3.5	11.0	2.9
	3	57.2	53.2	52.6	0.6	4.0	5.6	1.5
	15	283.7	252.5	239.4	13.1	31.2	25.4	6.7
	24	377.1	335.9	320.1	15.8	41.2	51.0	14.5

TABLE II—*Concluded.*

Day of experiment.	Length of period.	Total S.	Total SO ₄ -S.	Inorganic SO ₄ -S.	Ethereal SO ₄ -S.	Organic S.	Cystine.	Cystine. S.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
9	6	20.4	17.9	17.1	0.8	2.5	5.2	1.4
	18	117.0	113.1	106.1	7.0	3.9	11.7	3.1
	24	137.4	131.0	123.2	7.8	6.4	16.9	4.5
	24	128.8	107.1	99.4	7.7	14.7	16.5	4.4

* Cystine 0.812 gm. (sulfur = 0.218 gm.) given subcutaneously as the sodium salt; 50 cc. of water by mouth.

† Cystine 1.244 gm. (sulfur = 0.332 gm.) given subcutaneously as the sodium salt; 50 cc. of water by mouth.

excretion of urinary sulfates was also much depressed in subsequent periods. The urine obtained next morning contained protein and casts, evidence of definite kidney injury. During the first 24 hours following the injection, the total sulfur excretion was increased about 13 mg., but almost 60 per cent of the total sulfur was eliminated in the 3 hour period immediately following the administration of the cystine, for the most part as organic sulfur. The amount of oxidized (sulfate) sulfur excreted was also increased about 3-fold in this same 3 hour period. In this case, as in many others, examination of the analytical results of a single 24 hour period fails to reveal the true picture; *i.e.*, overwhelming of the oxidative function, followed by a depression of the normal excretion. In the succeeding 24 hours, excretion of oxidized sulfur was increased above the normal, so that the extra sulfur of the 48 hour period after injection of cystine accounted for about 27 per cent of the cystine sulfur administered. This represents the lowest rate of excretion and the lowest recovery of sulfur after cystine injection which we have observed in these experiments.

In marked contrast with these results are the data of a second experiment carried out nearly 2 months later on the same rabbit, after apparent recovery from the kidney injury, as evidenced by the absence of protein and casts from the urinē. Approximately the same amount of cystine was given as earlier but the administration as the sodium salt was oral. It will be noted (Table I) that

TABLE III.

Partition of Sulfur in Urine after Administration of Cystine and Cystine Diethyl Ester Hydrochloride.

Rabbit 17; weight 2.4 kilos.

Day of experiment.	Length of period.	Creatinine.	Total S.	Total SO ₄ -S.	Inorganic SO ₄ -S.	Ethereal SO ₄ -S.	Organic S.	Cystine.	Cystine S.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
1	8	28.9	22.7	20.1	19.1	1.0	2.6	5.0	1.3
	16	68.2	50.4	40.8	34.1	6.7	9.6	7.3	2.0
	24	97.1	73.1	60.9	53.2	7.7	12.2	12.3	3.3
2	8	32.9	16.1	12.3	9.6	2.7	3.7	6.3	1.7
	16	68.3	105.1	85.2	76.0	9.2	19.9	13.0	3.5
	24	101.2	121.2	97.5	85.6	11.9	23.6	19.3	5.2
3	4*	17.3	18.1	14.7	14.5	0.2	3.4	17.3	4.6
	4	18.0	53.8	45.4	45.2	0.2	8.4	7.6	2.0
	4	19.7	60.1	54.0	53.5	0.5	6.1	5.0	1.3
	12	51.8	134.3	117.2	110.5	6.7	17.1		
	24	106.8	266.3	231.3	223.7	7.6	35.0		
4	8	31.3	42.4	40.4	35.1	5.3	2.0	6.8	1.8
	16	50.2	131.2	111.6	102.0	9.6	19.6		
	24	81.5	173.6	152.0	137.1	14.9	21.6		
5	24	91.5	115.7	106.9	96.3	10.6	8.8	6.3	1.7
6	24	89.6	91.0	60.8	59.4	1.4	30.2	12.4	3.3
7	24	87.0	84.7	71.6	64.7	6.9	13.1	24.4	6.5
8	4†	15.2	29.9	24.7	23.7	1.0	5.2	7.3	1.9
	4	17.1	75.3	57.4	56.7	0.7	17.9	11.6	3.2
	4	14.7	71.8	59.6	58.7	0.9	12.2	6.4	1.7
	12	42.4	146.5	125.9	120.9	5.2	20.6	13.2	3.5
	24	89.4	323.5	267.6	260.0	7.6	55.9	38.4	10.4
9	8	28.8	43.0	40.2	37.7	2.5	2.8	8.9	2.3
	16	64.4	90.0	81.8	71.1	10.7	8.2	14.2	3.8
	24	93.2	133.0	122.0	108.8	13.2	11.0	23.1	6.1
10	8	28.2	26.1	23.8	19.6	4.2	2.3	7.3	2.0
	16	67.4	83.8	76.2	69.6	6.6	7.6	20.2	5.4
	24	95.6	109.9	100.0	89.2	10.8	9.9	27.5	7.4

TABLE III—*Concluded.*

Day of experiment.	Length of period.	Creatinine.	Total S.	Total SO ₄ -S.	Inorganic SO ₄ -S.	Ethereal SO ₄ -S.	Organic S.	Cystine.	Cystine S.
	<i>hrs.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
11	4†	13.6	18.3	15.0	14.6	0.4	3.3	7.8	2.1
	4	22.5	76.3	67.8	66.0	1.8	8.5	8.5	2.3
	4	15.7	61.5	57.4	56.6	0.8	4.1	7.5	2.0
	12	46.3	112.7	101.8	92.8	9.0	10.9	12.3	3.3
	24	98.1	268.8	242.0	230.0	12.0	26.8	36.1	9.7
12	8	24.7	35.4	33.0	29.7	3.3	2.4	8.9	2.4
	16	61.7	84.7	74.8	68.2	6.6	10.1	14.8	2.9
	24	86.4	120.1	107.8	97.9	9.9	12.5	23.7	5.3
13	24	95.4	108.7	77.6	74.3	3.3	31.1	17.6	4.7
14	24	84.6	130.4	90.7	85.0	5.7	39.7	20.3	5.4

* Cystine 1.0 gm. (sulfur = 0.267 gm.) given by mouth, suspended in 50 cc. of water.

† Cystine diethyl ester hydrochloride 1.689 gm. (sulfur = 0.267 gm.) given by mouth, in solution in 50 cc. of water.

‡ Cystine 1.0 gm. (sulfur = 0.267 gm.) given by mouth as the sodium salt dissolved in 50 cc. of water.

the amount of cystine eliminated as such in the urine (as indicated both by the direct colorimetric determination and by the organic sulfur) was but little increased over the normal amount, while the urinary sulfates increased notably even in the first 6 hours after ingestion. Cystine fed by mouth was absorbed at such a rate that oxidation was normal, the kidneys were not flooded with cystine, and there was no kidney injury as far as could be determined by the microscopic examination for casts and the absence of a positive test for protein. In this experiment the "extra" sulfur eliminated (almost entirely in the form of sulfates) in the first 24 hours following cystine feeding was about 77 per cent of that ingested, with only a small amount of "extra" sulfur (equivalent to about 8 per cent of the cystine fed), an amount within range of normal variations in sulfur excretion, in the succeeding 24 hour period.

Rabbit 16 (Table II) received cystine subcutaneously twice in smaller amounts than in the experiments just discussed. The

products of metabolism were excreted promptly, especially after the smaller amount, which was wholly eliminated in the first 24 hours (approximately 106 per cent of "extra" sulfur), only about 10 per cent appearing as cystine in the urine although there was a notable increase in the elimination of organic sulfur. Despite the larger amount of cystine introduced in the second experiment with this animal, the urinary cystine was only half of that found after the injection of the small dose and the change in the organic sulfur of the urine as well as the cystine sulfur was less marked. This, considered with the less rapid increase in the oxidized (sulfate) sulfur after the injection of the larger amount of cystine, would indicate a less rapid absorption from the site of injection. The absolute amounts of oxidized sulfur excreted in the first 24 hours were approximately the same in both experiments, though the percentages of recovery were different as the amounts injected were not equal.

The variations in the excretion of unchanged cystine noted in Tables I and II were similar to those obtained in all other experiments in which cystine was administered subcutaneously. When cystine was given by mouth, although there were individual variations in the rate of oxidation, in no case was there any considerable increase in the amount of cystine in the urine, the total excretion for 24 hours as measured by the Looney method rarely exceeding 35 mg. It would appear that unless the system is flooded with cystine, very little escapes through the kidney and that when the normal path of ingestion (oral) is followed, either the rate of absorption is slow or the cystine is taken up by the tissues so rapidly that the amount passing through the kidneys at any one time is not sufficiently great to cause excretion. That such excretion may readily occur when the amount of circulating cystine exceeds the threshold value of the kidney has been demonstrated repeatedly in unpublished experiments by one of us (L.) with Eisler and Lough. After the intravenous injection of 3 gm. of cystine (sodium salt) in Ringer-Locke solution into the marginal ear vein of a 2.6 kilo rabbit, 1.01 gm. of cystine had been excreted in an hour. Similarly 2 gm. of cystine were injected into the femoral vein of a 3 kilo rabbit. Within 10 minutes, the concentration of cystine was such that, on cooling, the urine deposited crystals of cystine. At the end of 30 minutes 507 mg.

of cystine had been excreted in the urine. With smaller amounts of cystine excretion also occurred. Thus, 460 mg. of cystine were excreted in the urine 45 minutes after the injection of 1.04 gm. of cystine into the marginal ear vein of a 2.15 kilo rabbit. Other experiments showed similarly the excretion of large amounts of cystine after intravenous injection.

Rabbit 17 (Table III) was fed cystine twice and also a more soluble form of cystine, cystine diethyl ester hydrochloride. The latter appeared to be absorbed somewhat more rapidly than the cystine, as the excretion during the first 4 hours after feeding was definitely higher than in the cystine experiment. The rate of excretion was greatest during the second and third 4 hour periods, being nearly constant during these 8 hours and reaching a value 3 to 4 times the normal value. The "extra" sulfur excreted within the first 24 hour period was equivalent to 60.9 and 61.6 per cent respectively of the ingested cystine in the two experiments in which cystine was fed. When cystine was fed in suspension rather than as the sodium salt, the elimination of "extra" sulfur continued in the second 24 hour period so that a total of 86.8 per cent of the sulfur of the ingested cystine appeared in the urine within 48 hours. When the cystine was fed as the sodium salt, the total recovery in terms of sulfur was less, 67.5 per cent being excreted in 48 hours. When the ester was fed, the excretion as urinary "extra" sulfur in the 24 and 48 hour periods following feeding were 82.0 and 92.8 per cent respectively. Other experiments have also indicated a greater excretion of "extra" sulfur after the feeding of the more soluble ester. These experiments detailed in Tables I to III are typical of the results we have obtained in studying the rate of elimination and the distribution of sulfur following the administration of cystine.

A few studies were made of the rate of absorption of cystine from the intestine. A normal rabbit of about 2 kilos weight was killed after a fast of 3 days duration. The gastrointestinal canal was removed, the contents washed into a volumetric flask, the protein was precipitated by the addition of .10 per cent sodium tungstate and 0.67 N hydrochloric acid,¹ and the contents of the

¹ Hydrochloric acid was substituted for the usual sulfuric acid, in the hope that it might be possible to determine the partition of sulfur in these filtrates. The presence of variable amounts of hydrogen sulfide made this impracticable.

flask diluted to a liter and filtered. The amount of cystine as determined colorimetrically by the Folin-Looney method (after allowance for the color in the blank to which no sulfite was added) was 72.7 mg., equivalent to 19.4 mg. of sulfur. Rabbit 29 (2.5 kilos) was fed 2 gm. of cystine and was killed 6 hours later. 385 mg. of cystine were present in the alimentary tract, or about 20 per cent of the amount fed. 6 hours after the oral administration of 1 gm. of cystine to Rabbit 33 (1.75 kilos), 33.3 per cent of the ingested cystine was present in the contents of the gastrointestinal canal. In each of these cases, the odor of hydrogen sulfide was noticeable on acidification of the intestinal contents, but was not more marked than in the case of the normal animal which received no cystine. The absorption of cystine would not appear to have proceeded rapidly, particularly since these were all fasted animals and the cystine was fed as the soluble sodium salt.

Oxidation of cystine to sulfate must occur rapidly after absorption from the intestine, since under our experimental conditions, excretion of "extra" sulfate sulfur began within 3 hours with a maximum rate per hour at about the 8th hour after feeding. The rate of excretion increased markedly, frequently reaching 25 to 26 mg. per hour for total sulfur, as compared with a normal rate of 4 to 6 mg. per hour. This markedly increased excretion did not appear to cause acute kidney injury, in so far as could be determined by the examination of the urine for casts or protein, so long as the "extra" sulfur was excreted in oxidized form, but kidney injury was usually associated with any marked increase in the excretion of unchanged cystine.

Very little of the "extra" sulfate excreted was conjugated. By far the largest percentage of the increase was in the inorganic sulfate fraction. It will be noted that the cystine sulfur of the urine failed to account for all the organic sulfur excreted. The excess may have been due to thiosulfates² although qualitative tests on some of the urine samples failed to reveal their presence. The "extra" organic sulfur may also have originated from other intermediary products of cystine oxidation; *e.g.*, cysteic acid or taurine.

While the greater part of the "extra" sulfur was excreted during

² Blum (1) has stated that thiosulfates are not present in the urine of the *Herbivora*, although of common occurrence in the urine of the dog and cat.

the first 24 hours, the sulfur content of the urine of the second 24 hours following administration of cystine was often somewhat greater than normal. By the 3rd day, the excretion had returned to or was even lower than the normal figure. The increase in sulfur excretion the 2nd day after feeding as noted by Sherwin and his associates (11) was seldom observed by us. In only one instance, after the subcutaneous injection of cystine, was there any definite rise and this was a slight one. Sherwin noted that the urine of the 2nd day after feeding cystine derivatives sometimes contained less sulfur than the control days, which he believed might indicate a definite disturbance of body processes. He also considered that some of the sulfur excreted after the ingestion of cystine and cysteine compounds might be derived from body tissues. Inasmuch as detailed data are not available, it is difficult to interpret these facts, particularly in view of the marked variations in sulfur excretion in animals even on a constant diet.

It is believed that the results presented in this paper, typical of those obtained with a considerable number of animals, afford some explanation of the variations in sulfur elimination frequently observed after the administration of cystine. Sufficient emphasis has not been placed previously on the variations in the rate of absorption, on the possible injury to the kidneys, which might slow up the excretion (as in Table I of the present series) even though absorption and oxidation had occurred, or on the desulfurization of the cystine molecule by the action of the microorganisms of the intestine with the elimination of part of the hydrogen sulfide or mercaptan formed through the feces. A complete picture of the extent of oxidation of ingested cystine and the excretion of the products of oxidation, together with the time relationships involved, is possible only when all these factors, some difficult to evaluate, are considered.

SUMMARY.

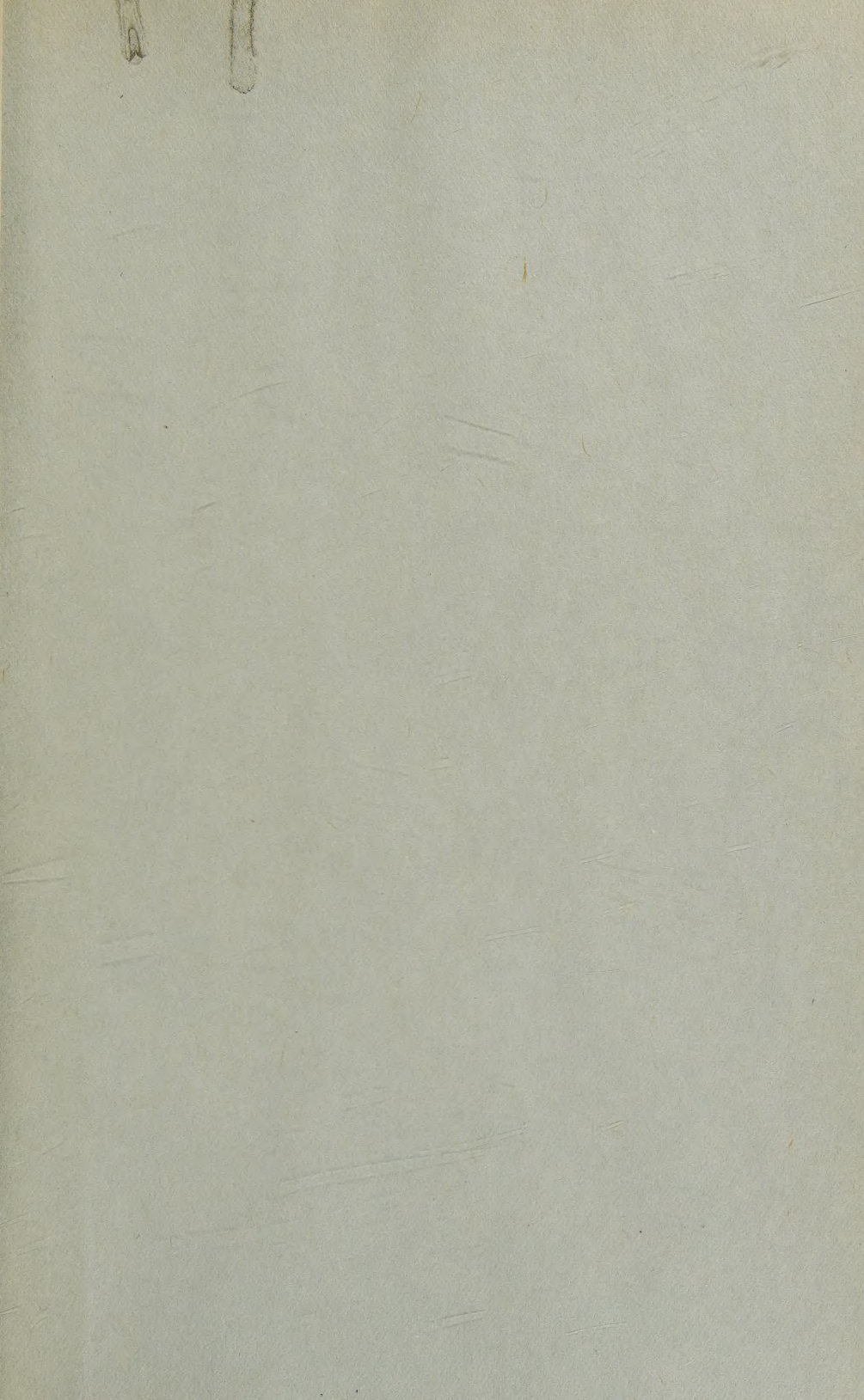
1. The absorption of moderate amounts of cystine from the alimentary canal of normal rabbits does not appear to occur with sufficient rapidity to overwhelm the oxidative capacity of the organism. The oxidation of the cystine absorbed and the excretion of the products of oxidation after oral administration proceed rapidly, from 60 to 85 per cent of the sulfur administered as

cystine being eliminated within 24 hours, largely as inorganic sulfates.

2. The course of the metabolism of cystine after subcutaneous injection is variable. If the cystine is rapidly absorbed from the site of injection, the effect is similar to that noted by Blum (1) when cystine was introduced directly into the peripheral circulation; *i.e.*, a considerable part of the cystine is excreted unchanged into the urine. This may be accompanied by kidney injury which may result in a diminished rate of excretion of the products of normal sulfur metabolism. If, however, the cystine is slowly absorbed from the site of injection, the course of the oxidation and excretion of the sulfur is very similar to that observed when cystine is fed by mouth.

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